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(51) International Patent Classification <sup>6</sup> : C12N 15/52, 5/10, 1/21, 9/00, C12Q 1/68, C07K 16/40, A61K 38/53		A1	(11) International Publication Number: WO 99/31252 (43) International Publication Date: 24 June 1999 (24.06.99)
(21) International Application Number: PCT/US98/25564 (22) International Filing Date: 2 December 1998 (02.12.98) (30) Priority Data: 08/989,289 12 December 1997 (12.12.97) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 08/989,289 (CIP) Filed on 12 December 1997 (12.12.97)		(74) Agents: BILLINGS, Lucy, J. et al.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
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(54) Title: UBIQUITIN-LIKE CONJUGATING PROTEIN

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      9      18      27      36      45      54
CG TTG CTG CGT TGT GAG GGG TGT CAG CTC AGT CCA TCC CAG GCA GCT CTT AGT

      63      72      81      90      99      108
GTG GAG CAG TGA ACT GTG TGT GGT TCC TTC TAC TTG GGG ATC ATG CAG AGA GCT
          M   Q   R   A

      117     126     135     144     153     162
TCG CGT CTG AAG AGA GAG CTG CAC ATG TTA GCC ACA GAG CCA CCC CCA GGC ATC
          S   R   L   K   R   E   L   H   M   L   A   T   E   P   P   P   G   I

      171     180     189     198     207     216
ACA TGT TGG CAA GAT AAA GAC CAA ATG GAT GAC CTG CGA GCT CAA ATA TTA GGT
          T   C   W   Q   D   K   D   Q   M   D   D   L   R   A   Q   I   L   G

      225     234     243     252     261     270
GGA GCC AAC ACA CCT TAT GAG AAA GGT GTT TTT AAG CTA GAA GTT ATC ATT CCT
          G   A   N   T   P   Y   E   K   G   V   F   K   L   E   V   I   I   P

      279     288     297     306     315     324
GAG AGG TAC CCA TTT GAA CCT CAG ATC CGA TTT CTC ACT CCA ATT TAT CAT
          E   R   Y   P   F   E   P   P   Q   I   R   F   L   T   P   I   Y   H

      333     342     351     360     369     378
CCA AAC ATT GAT TCT GCT GGA AGG ATT TGT CTG GAT GTT CTC AAA TTG CCA CCA
          P   N   I   D   S   A   G   R   I   C   L   D   V   L   K   L   P   P

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(57) Abstract

The invention provides a human ubiquitin-like conjugating protein (UBCLE) and polynucleotides which identify and encode UBCLE. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for treating or preventing disorders associated with expression of UBCLE.

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## UBIQUITIN-LIKE CONJUGATING PROTEIN

### TECHNICAL FIELD

5 This invention relates to nucleic acid and amino acid sequences of a ubiquitin-like conjugating protein and to the use of these sequences in the diagnosis, treatment, and prevention of cancer, and developmental, immune and neuronal disorders.

### BACKGROUND OF THE INVENTION

10 The ubiquitin conjugation system (UCS) plays a major role in the degradation of cellular proteins in eukaryotic cells and in some bacterial cells. UCS mediates the elimination of abnormal proteins and regulates the half-lives of other important regulatory proteins that control gene transcription and cell cycle progression. The UCS is reported to degrade mitotic cyclic kinases, oncoproteins, tumor suppressors, viral proteins, 15 transcriptional regulators, and receptors associated with signal transduction (Verma, R. et al. (1997) *Science* 278:455-460; Ciechanover, A. (1994) *Cell* 79:13-21).

Several steps are involved in ubiquitin (Ub) conjugation and protein degradation (Jentsch, S. (1992) *Annu. Rev. Genet.* 26:179-207). First, Ub, a small, heat stable protein, is activated by a ubiquitin-activating enzyme (E1). This ATP dependent activation 20 involves binding of the C-terminus of Ub to the thiol group of a cysteine residue of E1. Activated Ub is subsequently transferred to one of several Ub-conjugating enzymes (E2). Each E2 has a recognition subunit which allows it to interact with proteins carrying a particular degradation signal. E2 links the Ub molecule through its C-terminal glycine to an internal lysine of the target protein. Different ubiquitin-dependent proteolytic pathways 25 employ structurally similar, but distinct, E2s, and in some instances, accessory factors known as ubiquitin-ligases or E3s, are required to work in conjunction with E2s for recognition of certain substrates (Jensen, J. P. et al. (1995) *J. Biol. Chem.* 270:30408-30414). More than one Ub molecule may be needed to ubiquinate a target protein which is subsequently recognized and degraded by a proteasome. After degradation, Ub is released 30 and reutilized.

Prior to activation, Ub is usually expressed as a fusion protein composed of an

N-terminal ubiquitin and a C-terminal extension protein (CEP) or as a polyubiquitin protein with Ub monomers attached head to tail. CEPs have characteristics of a variety of regulatory proteins; most are highly basic, contain up to 30% lysine and arginine residues, and have nucleic acid-binding domains (Monia, B.P. et al. (1989) *J. Biol. Chem.* 264:4093-4103).

5 The fusion protein is an important intermediate which appears to mediate co-regulation of the cell's translational and protein degradation activities, as well as localization of the inactive enzyme to specific cellular sites. Once delivered, C-terminal hydrolases cleave the fusion protein to release a functional Ub (Monia et al., *supra*).

The E2s are important for substrate specificity in several UCS pathways. All E2s 10 have a conserved ubiquitin conjugation (UBC) domain of approximately 16 kD, at least 35% identity with each other, and contain a centrally located cysteine residue which is necessary for ubiquitin-enzyme thiolester formation (Jentsch, *supra*). A highly conserved proline-rich element is located N-terminal to the active cysteine residue. Structural variations outside of this conserved domain are used to separate the E2 enzymes into 15 classes. The E2s of class 1 (E2-1) consist of the conserved UBC domain and include yeast E2-1 and UBCs 4, 5, and 7. These E2s are thought to require E3 to carry out their activities (Jentsch, *supra*). UBC7 has been shown to recognize ubiquitin as a substrate and to form polyubiquitin chains *in vitro* (van Nocker, S. et al. (1996) *J. Biol. Chem.* 271:12150-58). E2s of class 2 (E2-2) have various unrelated C-terminal extensions that 20 contribute to substrate specificity and cellular localization. The yeast E2-2 enzymes, UBC2 and UBC3, have highly acidic C-terminal extensions that promote interactions with basic substrates such as histones. Yeast UBC6 has a hydrophobic signal-anchor sequence that localizes the protein to the endoplasmic reticulum.

Abnormal activities of the UCS are implicated in a number of diseases and 25 disorders. These include, e.g., cachexia (Llovera, M. et al. (1995) *Int. J. Cancer* 61: 138-141), degradation of the tumor-suppressor protein, p53 (Ciechanover, *supra*), and neurodegeneration such as observed in Alzheimer's disease (Gregori, L. et al. (1994) *Biochem. Biophys. Res. Commun.* 203: 1731-1738). Since ubiquitin conjugation is a rate-limiting step in antigen presentation, the ubiquitin degradation pathway may also have 30 a critical role in the immune response (Grant E.P. et al. (1995) *J. Immunol.* 155: 3750-3758).

The discovery of new ubiquitin-conjugating-like protein and the polynucleotides

encoding it satisfies a need in the art by providing new compositions which are useful in the diagnosis, treatment, and prevention of cancer, and developmental, immune and neuronal disorders.

5

## SUMMARY OF THE INVENTION

The invention features a substantially purified polypeptide, ubiquitin-like conjugating protein (UBCLE), comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention further provides a substantially purified variant of UBCLE having at 10 least 90% amino acid identity to the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1. The invention also provides an isolated and purified polynucleotide sequence encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1. The invention also includes an isolated and purified polynucleotide variant having at least 90% polynucleotide identity to the polynucleotide 15 sequence encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

Additionally, the invention provides a composition comprising a polynucleotide sequence encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1. The invention further provides an isolated and purified 20 polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, as well as an isolated and purified polynucleotide sequence which is complementary to the polynucleotide sequence encoding the 25 polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention also provides an isolated and purified polynucleotide sequence comprising SEQ ID NO:2 or a fragment of SEQ ID NO:2, and an isolated and purified polynucleotide variant having at least 90% polynucleotide identity to the polynucleotide sequence comprising SEQ ID NO:2 or a fragment of SEQ ID NO:2. The invention also 30 provides an isolated and purified polynucleotide sequence which is complementary to the polynucleotide sequence comprising SEQ ID NO:2 or a fragment of SEQ ID NO:2.

The invention further provides an expression vector containing at least a fragment

of the polynucleotide sequence encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1. In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide comprising the 5 amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, the method comprising the steps of: (a) culturing the host cell containing an expression vector containing at least a fragment of a polynucleotide sequence encoding UBCLE under conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture.

10 The invention also provides a pharmaceutical composition comprising a substantially purified UBCLE having the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1 in conjunction with a suitable pharmaceutical carrier.

The invention further includes a purified antibody which binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, as 15 well as a purified agonist and a purified antagonist of the polypeptide.

The invention also provides a method for treating or preventing a cancer, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of UBCLE.

The invention also provides a method for treating or preventing an developmental 20 disorder, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of UBCLE.

The invention also provides a method for treating or preventing an immune disorder, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of UBCLE.

25 The invention also provides a method for treating or preventing a neuronal disorder, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of UBCLE.

The invention also provides a method for detecting a polynucleotide encoding UBCLE in a biological sample containing nucleic acids, the method comprising the steps 30 of: (a) hybridizing the complement of the polynucleotide sequence encoding the polypeptide comprising SEQ ID NO:1 or a fragment of SEQ ID NO:1 to at least one of the nucleic acids of the biological sample, thereby forming a hybridization complex; and (b)

detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide encoding UBCLE in the biological sample. In one aspect, the nucleic acids of the biological sample are amplified by the polymerase chain reaction prior to the hybridizing step.

5

### BRIEF DESCRIPTION OF THE FIGURES

Figures 1A, 1B, and 1C show the amino acid sequence (SEQ ID NO:1) and nucleic acid sequence (SEQ ID NO:2) of UBCLE. The alignment was produced using MacDNASIS PRO™ software (Hitachi Software Engineering Co. Ltd., San Bruno, CA).

10 Figure 2 shows the amino acid sequence alignments between UBCLE (2501808; SEQ ID NO:1) and UBCH5C (GI 1145691; SEQ ID NO:3), produced using the multisequence alignment program of DNASTAR™ software (DNASTAR Inc, Madison WI).

15

### DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, 20 and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a 25 reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those 30 described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, vectors, and

methodologies which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

## 5 DEFINITIONS

"UBCLE," as used herein, refers to the amino acid sequences of substantially purified UBCLE obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and preferably the human species, from any source, whether natural, synthetic, semi-synthetic, or recombinant.

10 The term "agonist," as used herein, refers to a molecule which, when bound to UBCLE, increases or prolongs the duration of the effect of UBCLE. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of UBCLE.

An "allele" or an "allelic sequence," as these terms are used herein, is an 15 alternative form of the gene encoding UBCLE. Alleles may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give 20 rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding UBCLE, as described herein, include 25 those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide the same UBCLE or a polypeptide with at least one functional characteristic of UBCLE. Included within this definition are polymorphisms 30 which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding UBCLE, and improper or unexpected hybridization to alleles, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding UBCLE. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent UBCLE. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or

the amphipathic nature of the residues, as long as the biological or immunological activity of UBCLE is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups having similar hydrophilicity values

5 may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

The terms "amino acid" or "amino acid sequence," as used herein, refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. In this context, "fragments" refers to

10 fragments of UBCLE which are preferably about 5 to about 15 amino acids in length and which retain some biological activity or immunological activity of UBCLE. Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the

15 recited protein molecule.

"Amplification," as used herein, relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art. (See, e.g., Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, 20 NY.)

The term "antagonist," as it is used herein, refers to a molecule which, when bound to UBCLE, decreases the amount or the duration of the effect of the biological or immunological activity of UBCLE. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or any other molecules which decrease the effect of UBCLE.

25 As used herein, the term "antibody" refers to intact molecules as well as to fragments thereof, such as Fa, F(ab')<sub>2</sub>, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind UBCLE polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a

30 mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and

keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant," as used herein, refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein 5 or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (given regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

10 The term "antisense," as used herein, refers to any composition containing a nucleic acid sequence which is complementary to a specific DNA or RNA sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense molecules may be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides 15 combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" can refer to the antisense strand, and the designation "positive" can refer to the sense strand.

As used herein, the term "biologically active," refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, 20 "immunologically active" refers to the capability of the natural, recombinant, or synthetic UBCLE, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" or "complementarity," as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by 25 base pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and 30 strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" or a "composition comprising a given amino acid sequence," as these terms are used herein, refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions 5 comprising polynucleotide sequences encoding UBCLE or fragments of UBCLE may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, 10 etc.).

The phrase "consensus sequence," as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using XL-PCR™ (Perkin Elmer, Norwalk, CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte Clone using a 15 computer program for fragment assembly, such as the GELVIEW™ Fragment Assembly system (GCG, Madison, WI). Some sequences have been both extended and assembled to produce the consensus sequence .

As used herein, the term "correlates with expression of a polynucleotide" indicates that the detection of the presence of nucleic acids, the same or related to a nucleic acid 20 sequence encoding UBCLE, by northern analysis is indicative of the presence of nucleic acids encoding UBCLE in a sample, and thereby correlates with expression of the transcript from the polynucleotide encoding UBCLE.

A "deletion," as the term is used herein, refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or 25 nucleotides.

The term "derivative," as used herein, refers to the chemical modification of UBCLE, of a polynucleotide sequence encoding UBCLE, or of a polynucleotide sequence complementary to a polynucleotide sequence encoding UBCLE. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an 30 alkyl, acyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process

that retains at least one biological or immunological function of the polypeptide from which it was derived.

The term "homology," as used herein, refers to a degree of complementarity. There may be partial homology or complete homology. The word "identity" may 5 substitute for the word "homology." A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization, and the like) under conditions of 10 reduced stringency. A substantially homologous sequence or hybridization probe will compete for and inhibit the binding of a completely homologous sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a 15 selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% homology or identity). In the absence of non-specific binding, the substantially homologous sequence or probe will not hybridize to the second non-complementary target sequence.

20 "Human artificial chromosomes" (HACs), as described herein, are linear microchromosomes which may contain DNA sequences of about 10 K to 10 M in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance. (Harrington, J.J. et al. (1997) *Nat Genet.* 15:345-355.)

The term "humanized antibody," as used herein, refers to antibody molecules in 25 which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization," as the term is used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

30 As used herein, the term "hybridization complex" as used herein, refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed

in solution (e.g.,  $C_0t$  or  $R_0t$  analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

5 The words "insertion" or "addition," as used herein, refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, to the sequence found in the naturally occurring molecule.

The term "microarray," as used herein, refers to an array of distinct polynucleotides or oligonucleotides arrayed on a substrate, such as paper, nylon or any other type of 10 membrane, filter, chip, glass slide, or any other suitable solid support.

The term "modulate," as it appears herein, refers to a change in the activity of UBCLE. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of 15 UBCLE.

15 The phrases "nucleic acid" or "nucleic acid sequence," as used herein, refer to an oligonucleotide, nucleotide, polynucleotide, or any fragment thereof, to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material. In this context, "fragments" refers to those nucleic acid 20 sequences which are greater than about 60 nucleotides in length, and most preferably are at least about 100 nucleotides, at least about 1000 nucleotides, or at least about 10,000 nucleotides in length.

The term "oligonucleotide," as used herein, refers to a nucleic acid sequence of at least about 6 nucleotides to 60 nucleotides, preferably about 15 to 30 nucleotides, and 25 most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in a hybridization assay or microarray. As used herein, the term "oligonucleotide" is substantially equivalent to the terms "amplimers," "primers," "oligomers," and "probes," as these terms are commonly defined in the art.

"Peptide nucleic acid" (PNA), as used herein, refers to an antisense molecule or 30 anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary

single stranded DNA and RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell. (Nielsen, P.E. et al. (1993) *Anticancer Drug Des.* 8:53-63.

The term "sample," as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acids encoding UBCLE, or fragments thereof, or 5 UBCLE itself may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA (in solution or bound to a solid support); a tissue; a tissue print; and the like.

As used herein, the terms "specific binding" or "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The 10 interaction is dependent upon the presence of a particular structure of the protein recognized by the binding molecule (i.e., the antigenic determinant or epitope). For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

15 As used herein, the term "stringent conditions" refers to conditions which permit hybridization between polynucleotide sequences and the claimed polynucleotide sequences. Suitably stringent conditions can be defined by, for example, the concentrations of salt or formamide in the prehybridization and hybridization solutions, or by the hybridization temperature, and are well known in the art. In particular, stringency 20 can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

For example, hybridization under high stringency conditions could occur in about 50% formamide at about 37°C to 42°C. Hybridization could occur under reduced stringency conditions in about 35% to 25% formamide at about 30°C to 35°C. In 25 particular, hybridization could occur under high stringency conditions at 42°C in 50% formamide, 5X SSPE, 0.3% SDS, and 200 µg/ml sheared and denatured salmon sperm DNA. Hybridization could occur under reduced stringency conditions as described above, but in 35% formamide at a reduced temperature of 35°C. The temperature range corresponding to a particular level of stringency can be further narrowed by calculating the 30 purine to pyrimidine ratio of the nucleic acid of interest and adjusting the temperature accordingly. Variations on the above ranges and conditions are well known in the art.

The term "substantially purified," as used herein, refers to nucleic acid or amino

acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

5 A "substitution," as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Transformation," as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or 10 eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host 15 chromosome, and refers to cells which transiently express the inserted DNA or RNA for limited periods of time.

A "variant" of UBCLE, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., 20 replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs 25 well known in the art, for example, DNASTAR software.

#### THE INVENTION

The invention is based on the discovery of a new human ubiquitin-like conjugating protein (UBCLE), the polynucleotides encoding UBCLE, and the use of these 30 compositions for the diagnosis, treatment, or prevention of cancer, and developmental, immune, and neuronal disorders.

Nucleic acids encoding the UBCLE of the present invention were first identified in

Incyte Clone 2501808 from the adrenal tumor cDNA library (ADRETUT05) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:2, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 2501808 (ADRETUT05), 291812 (TMLR3DT01), and 743853

5 (BRAITUT01).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1. UBCLE is 198 amino acids in length and has a potential ubiquitin conjugating enzyme active site between residues Y<sub>75</sub> and V<sub>89</sub>, a potential N-glycosylation site at residue N<sub>176</sub>, a potential cAMP phosphorylation site at residue R<sub>181</sub>, a potential casein kinase II phosphorylation site at residue T<sub>44</sub>, and two potential protein kinase C phosphorylation sites at residues T<sub>147</sub> and T<sub>178</sub>. As shown in Figure 2, UBCLE has chemical and structural homology with UBCH5C (GI 1145691; SEQ ID NO:3). In particular, UBCLE and UBCH5C share 32% sequence identity. Northern analysis shows the expression of this sequence in various cDNA libraries, at least 10 52% of which are immortalized or cancerous, at least 39% of which involve cell 15 proliferation. Of particular note is the expression in nervous system tissues.

The invention also encompasses UBCLE variants. A preferred UBCLE variant is one having at least about 80%, more preferably at least about 90%, and most preferably at least about 95% amino acid sequence identity to the UBCLE amino acid sequence.

20 The invention also encompasses polynucleotides which encode UBCLE. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising the sequence of SEQ ID NO:2, which encodes an UBCLE.

The invention also encompasses a variant of a polynucleotide sequence encoding UBCLE. In particular, such a variant polynucleotide sequence will have at least about 25 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding UBCLE. A particular aspect of the invention encompasses a variant of SEQ ID NO:2 which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to SEQ ID NO:2.

30 It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding UBCLE, some bearing minimal homology to the polynucleotide sequences of any known and naturally occurring

gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring 5 UBCLE, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode UBCLE and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring UBCLE under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding UBCLE or its derivatives possessing a 10 substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding UBCLE and its derivatives without altering the encoded amino acid sequences include the production of 15 RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode UBCLE and UBCLE derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available 20 expression vectors and cell systems using reagents that are well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding UBCLE or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown 25 in SEQ ID NO:2, or a fragment of SEQ ID NO:2, under various conditions of stringency as taught in Wahl, G.M. and S.L. Berger (1987; Methods Enzymol. 152:399-407) and Kimmel, A.R. (1987; Methods Enzymol. 152:507-511).

Methods for DNA sequencing are well known and generally available in the art and may be used to practice any of the embodiments of the invention. The methods may 30 employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp., Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of polymerases and proofreading

exonucleases such as those found in the ELONGASE Amplification System marketed by GIBCO/BRL (Gaithersburg, MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI Catalyst and 373 and 377 DNA

5 Sequencers (Perkin Elmer).

The nucleic acid sequences encoding UBCL<sub>E</sub> may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal primers to retrieve unknown 10 sequence adjacent to a known locus. (Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) In particular, genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an 15 appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region. (Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) The primers may be designed using commercially available software such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, MN) or another 20 appropriate program to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to 72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

25 Another method which may be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA. (Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to place an engineered double-stranded sequence into an unknown fragment of the 30 DNA molecule before performing PCR. Another method which may be used to retrieve unknown sequences is that of Parker, J.D. et al. (1991; Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries to walk

genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable 5 in that they will include more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used 10 to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software 15 (e.g., Genotyper™ and Sequence Navigator™, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments 20 thereof which encode UBCLE may be used in recombinant DNA molecules to direct expression of UBCLE, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced, and these sequences may be used to clone and express UBCLE.

25 As will be understood by those of skill in the art, it may be advantageous to produce UBCLE-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript 30 generated from the naturally occurring sequence.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter UBCLE encoding sequences for a

variety of reasons including, but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, site-directed mutagenesis may 5 be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding UBCLE may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of UBCLE activity, 10 it may be useful to encode a chimeric UBCLE protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the UBCLE encoding sequence and the heterologous protein sequence, so that UBCLE may be cleaved and purified away from the heterologous moiety.

15 In another embodiment, sequences encoding UBCLE may be synthesized, in whole or in part, using chemical methods well known in the art. (See Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, and Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232.) Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of UBCLE, or a fragment thereof. For 20 example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J.Y. et al. (1995) Science 269:202-204) and automated synthesis may be achieved using the ABI 431A Peptide Synthesizer (Perkin Elmer).

The newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography. (See, for example, Creighton, T. (1983) Proteins. 25 Structures and Molecular Principles, WH Freeman and Co., New York, NY.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, for example, the Edman degradation procedure described in Creighton, supra.) Additionally, the amino acid sequence of UBCLE, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any 30 part thereof, to produce a variant polypeptide.

In order to express a biologically active UBCLE, the nucleotide sequences encoding UBCLE or derivatives thereof may be inserted into appropriate expression

vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding UBCLE and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. et al. (1989; Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, NY) and Ausubel, F.M. et al. (1989; Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY).

10 A variety of expression vector/host systems may be utilized to contain and express sequences encoding UBCLE. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed 15 with virus expression vectors (e.g., cauliflower mosaic virus (CaMV) or tobacco mosaic virus (TMV)) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

The invention is not limited by the host cell employed.

The "control elements" or "regulatory sequences" are those non-translated regions 20 of the vector (i.e., enhancers, promoters, and 5' and 3' untranslated regions) which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, 25 inducible promoters such as the hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, La Jolla, CA) or pSport1™ plasmid (GIBCO/BRL), and the like, may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO, and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) may 30 be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding UBCLE, vectors based on SV40 or

EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for UBCLE. For example, when large quantities of UBCLE are needed for the induction of antibodies, vectors which direct high level expression of fusion 5 proteins that are readily purified may be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as Bluescript® (Stratagene), in which the sequence encoding UBCLE may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced, pIN vectors (Van Heeke, G. and S.M. Schuster (1989) J. 10 Biol. Chem. 264:5503-5509), and the like. pGEX vectors (Promega, Madison, WI) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include 15 heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH, may be used. For reviews, see Ausubel (*supra*) and Grant et al. (1987; Methods Enzymol. 153:516-544).

20 In cases where plant expression vectors are used, the expression of sequences encoding UBCLE may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV. (Takamatsu, N. (1987) EMBO J. 6:307-311.) Alternatively, plant promoters such as the small subunit of RUBISCO or 25 heat shock promoters may be used. (Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews. (See, for example, Hobbs, S. or Murry, L.E. in 30 McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.)

An insect system may also be used to express UBCLE. For example, in one such

system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding UBCLE may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful 5 insertion of UBCLE will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which UBCLE may be expressed. (Engelhard, E.K. et al. (1994) Proc. Nat. Acad. Sci. 91:3224-3227.)

In mammalian host cells, a number of viral-based expression systems may be 10 utilized. In cases where an adenovirus is used as an expression vector, sequences encoding UBCLE may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing UBCLE in infected host cells. (Logan, J. and T. Shenk (1984) Proc. Natl. 15 Acad. Sci. 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) may also be employed to deliver larger 20 fragments of DNA than can be contained and expressed in a plasmid. HACs of about 6 M to 10 M are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding UBCLE. Such signals include the ATG initiation codon and adjacent 25 sequences. In cases where sequences encoding UBCLE and its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert.

Exogenous translational elements and initiation codons may be of various origins, both 30 natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular cell system used, such as those described in the literature. (Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding, and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC, Bethesda, MD) and may be chosen to ensure the correct modification and processing of the foreign protein.

For long term, high yield production of recombinant proteins, stable expression is preferred. For example, cell lines capable of stably expressing UBCLE can be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase genes (Wigler, M. et al. (1977) Cell 11:223-32) and adenine phosphoribosyltransferase genes (Lowy, I. et al. (1980) Cell 22:817-23), which can be employed in *tk* or *apr* cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-70); *npt* confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14); and *als* or *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, *trpB*, which allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine. (Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl.

Acad. Sci. 85:8047-51.) Recently, the use of visible markers has gained popularity with such markers as anthocyanins,  $\beta$  glucuronidase and its substrate GUS, and luciferase and its substrate luciferin. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a 5 specific vector system. (Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding UBCLE is inserted within a marker gene sequence, transformed cells containing sequences encoding UBCLE can be identified by the absence 10 of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding UBCLE under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain the nucleic acid sequence encoding UBCLE 15 and express UBCLE may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

20 The presence of polynucleotide sequences encoding UBCLE can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding UBCLE. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences encoding UBCLE to detect transformants containing DNA or RNA encoding UBCLE.

25 A variety of protocols for detecting and measuring the expression of UBCLE, using either polyclonal or monoclonal antibodies specific for the protein, are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two 30 non-interfering epitopes on UBCLE is preferred, but a competitive binding assay may be employed. These and other assays are well described in the art, for example, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul, MN) and

in Maddox, D.E. et al. (1983; J. Exp. Med. 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to 5 polynucleotides encoding UBCLE include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding UBCLE, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase 10 such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Pharmacia & Upjohn (Kalamazoo, MI), Promega (Madison, WI), and U.S. Biochemical Corp. (Cleveland, OH). Suitable reporter molecules or labels which may be used for ease of detection include 15 radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding UBCLE may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or contained 20 intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode UBCLE may be designed to contain signal sequences which direct secretion of UBCLE through a prokaryotic or eukaryotic cell membrane. Other constructions may be used to join sequences encoding UBCLE to nucleotide sequences encoding a polypeptide domain 25 which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences, such as those specific for Factor XA or 30 enterokinase (Invitrogen, San Diego, CA), between the purification domain and the UBCLE encoding sequence may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing UBCLE and a nucleic acid

encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on immobilized metal ion affinity chromatography (IMAC; described in Porath, J. et al. (1992) *Prot. Exp. Purif.* 3: 263-281)), while the enterokinase cleavage site provides a means for purifying UBCLE from 5 the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. et al. (1993; *DNA Cell Biol.* 12:441-453).

Fragments of UBCLE may be produced not only by recombinant production, but also by direct peptide synthesis using solid-phase techniques. (Merrifield J. (1963) *J. Am. Chem. Soc.* 85:2149-2154.) Protein synthesis may be performed by manual techniques or 10 by automation. Automated synthesis may be achieved, for example, using the Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Various fragments of UBCLE may be synthesized separately and then combined to produce the full length molecule.

#### THERAPEUTICS

15 Chemical and structural homology exists between UBCLE and human UBCH5C (GI 1145690). In addition, UBCLE is expressed in cancer, and developmental, immune, and neuronal disorders where UBCLE plays a role in the cell cycle and in cell signaling. Therefore, UBCLE appears to play a role in cancer, and developmental, immune, and neuronal disorders..

20 Degradation of tumor suppressor proteins such as p53 by E2 enzymes may contribute to the development of cancer. Therefore, in one embodiment, an antagonist of UBCLE may be administered to a subject to treat or prevent a cancer. Such cancers can include, but are not limited to, adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, 25 bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

30 In an additional embodiment, a vector expressing the complement of the polynucleotide encoding UBCLE may be administered to a subject to treat or prevent a cancer including, but not limited to, those described above.

Abnormalities in processing of neural proteins (AP) by enzymes of the UCS may contribute to neuronal disorders. Therefore, in another embodiment, an antagonist which

modulates the activity of UBCLE may be administered to a subject to treat or prevent a neuronal disorder. Such neuronal disorders can include, but are not limited to, akathesia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar disorder, catatonia, cerebral neoplasms, dementia, depression, Down's syndrome, tardive dyskinesia, 5 dystonias, epilepsy, Huntington's disease, multiple sclerosis, Parkinson's disease, paranoid psychoses, schizophrenia, and Tourette's disorder.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding UBCLE may be administered to a subject to treat or prevent a neuronal disorder including, but not limited to, those disorders described above.

10 In a further embodiment, an antagonist of UBCLE may be administered to a subject to treat or prevent a developmental disorder. Such developmental disorders may include, but are not limited to, renal tubular acidosis, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, gonadal dysgenesis, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, 15 hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Sydenham's chorea and cerebral palsy, spinal bifida, and congenital glaucoma, cataract, or sensorineural hearing loss.

20 In an additional embodiment, a vector expressing the complement of the polynucleotide encoding UBCLE may be administered to a subject to treat or prevent a developmental disorder including, but not limited to, those disorders described above.

In a further embodiment, an antagonist of UBCLE may be administered to a subject to treat or prevent an immune disorder. Such immune disorders may include, but are not limited to, Addison's disease, adult respiratory distress syndrome, allergies, 25 ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, 30 lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus,

systemic sclerosis, ulcerative colitis, Werner syndrome, and complications of cancer, hemodialysis, and extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and helminthic infections; and trauma.

In an additional embodiment, a vector expressing the complement of the 5 polynucleotide encoding UBCLE may be administered to a subject to treat or prevent an immune disorder including, but not limited to, those disorders described above.

In one aspect, an antibody which specifically binds UBCLE may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express UBCLE.

10 In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act 15 synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of UBCLE may be produced using methods which are generally known in the art. In particular, purified UBCLE may be used to produce antibodies or to 20 screen libraries of pharmaceutical agents to identify those which specifically bind UBCLE. Antibodies to UBCLE may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are especially 25 preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with UBCLE or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants 30 include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli

Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to UBCLE have an amino acid sequence consisting of at least about 5 amino acids, and, more preferably, of at least about 10 amino acids. It is also preferable that 5 these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of UBCLE amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

10 Monoclonal antibodies to UBCLE may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (Kohler, G. et al. (1975) *Nature* 256:495-497; Kozbor, D. et al. (1985) *J. Immunol. Methods* 81:31-42; Cote, R.J. et al. 15 (1983) *Proc. Natl. Acad. Sci.* 80:2026-2030; and Cole, S.P. et al. (1984) *Mol. Cell Biol.* 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (Morrison, S.L. 20 et al. (1984) *Proc. Natl. Acad. Sci.* 81:6851-6855; Neuberger, M.S. et al. (1984) *Nature* 312:604-608; and Takeda, S. et al. (1985) *Nature* 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce UBCLE-specific single chain antibodies. Antibodies with 25 related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (Burton D.R. (1991) *Proc. Natl. Acad. Sci.* 88:11120-11123.)

Antibodies may also be produced by inducing in vivo production in the 30 lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (Orlandi, R. et al. (1989) *Proc. Natl. Acad. Sci.* 86: 3833-3837, and Winter, G. et al. (1991) *Nature* 349:293-299.)

Antibody fragments which contain specific binding sites for UBCLE may also be generated. For example, such fragments include, but are not limited to, F(ab')2 fragments

produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (Huse, W.D. et al. (1989) *Science* 254:1275-1281.)

5        Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between UBCLE and its specific antibody. A two-site, monoclonal-based  
10      immunoassay utilizing monoclonal antibodies reactive to two non-interfering UBCLE epitopes is preferred, but a competitive binding assay may also be employed. (Maddox,  
15      supra.)

In another embodiment of the invention, the polynucleotides encoding UBCLE, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect,  
15      the complement of the polynucleotide encoding UBCLE may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding UBCLE. Thus, complementary molecules or fragments may be used to modulate UBCLE activity, or to achieve regulation of gene function. Such technology is now well known in the art,  
20      and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding UBCLE.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known  
25      to those skilled in the art can be used to construct vectors which will express nucleic acid sequence complementary to the polynucleotides of the gene encoding UBCLE. These techniques are described, for example, in Sambrook (supra) and in Ausubel (supra).

Genes encoding UBCLE can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide or fragment thereof  
30      encoding UBCLE. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous

nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by

5 designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding UBCLE. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the

10 ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY.) A complementary sequence or antisense molecule may also be designed to block translation

15 of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme

20 molecules specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding UBCLE.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of

25 between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

30 Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase

phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding UBCLE. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that 5 synthesize complementary RNA constitutively or inducibly can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather 10 than phosphodiester linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

15 Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in 20 the art, such as those described in Goldman, C.K. et al. (1997; Nature Biotechnology 15:462-466).

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

25 An additional embodiment of the invention relates to the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of UBCLE, antibodies to UBCLE, and mimetics, agonists, antagonists, or inhibitors of UBCLE. The compositions may be administered alone or in combination 30 with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient

alone, or in combination with other agents, drugs or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, 5 intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may 10 be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as 15 tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be 20 added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating 25 or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer 30 solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, 5 optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as 10 Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid 15 esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier 20 to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, 25 granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acid. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred 30 preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of UBCLE, such labeling would include amount, frequency, and method of administration.

5 Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially 10 either in cell culture assays of neoplastic cells, for example, or in animal models, usually mice, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for 15 example UBCLE or fragments thereof, antibodies of UBCLE, and agonists, antagonists or inhibitors of UBCLE, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED50 (the dose therapeutically effective in 50% of the population) or LD50 (the dose lethal to 50% of the population) 20 statistics. The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the LD50/ED50 ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations 25 that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide 30 sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, diet, time and frequency of

administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

5 Normal dosage amounts may vary from 0.1  $\mu$ g to 100,000  $\mu$ g, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or  
10 polypeptides will be specific to particular cells, conditions, locations, etc.

#### DIAGNOSTICS

In another embodiment, antibodies which specifically bind UBCLE may be used for the diagnosis of disorders characterized by expression of UBCLE, or in assays to  
15 monitor patients being treated with UBCLE or agonists, antagonists, and inhibitors of UBCLE. Antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for UBCLE include methods which utilize the antibody and a label to detect UBCLE in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be  
20 labeled by covalent or non-covalent joining with a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring UBCLE, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels  
25 of UBCLE expression. Normal or standard values for UBCLE expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to UBCLE under conditions suitable for complex formation. The amount of standard complex formation may be quantified by various methods, preferably by photometric means. Quantities of UBCLE expressed in subject,  
30 control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding UBCLE may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of UBCLE may be correlated with disease. The diagnostic assay may be used to distinguish between absence, presence, and excess expression of UBCLE, and to monitor regulation of UBCLE levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding UBCLE or closely related molecules may be used to identify nucleic acid sequences which encode UBCLE. The specificity of the probe, whether it is made from a highly specific region (e.g., the 5' regulatory region) or from a less specific region (e.g., the 3' coding region), and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), will determine whether the probe identifies only naturally occurring sequences encoding UBCLE, alleles, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably contain at least 50% of the nucleotides from any of the UBCLE encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:2 or from genomic sequences including promoter and enhancer elements and introns of the naturally occurring UBCLE.

Means for producing specific hybridization probes for DNAs encoding UBCLE include the cloning of polynucleotide sequences encoding UBCLE or UBCLE derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding UBCLE may be used for the diagnosis of a disorder associated with expression of UBCLE. Disorders include, but are not limited to, cancers such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone

marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; neuronal disorders such as akathesia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar disorder, catatonia, 5 cerebral neoplasms, dementia, depression, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, multiple sclerosis, Parkinson's disease, paranoid psychoses, schizophrenia, and Tourette's disorder; developmental disorders such as renal tubular acidosis, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, gonadal dysgenesis, myelodysplastic syndrome, hereditary 10 mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Sydenham's chorea and cerebral palsy, spinal bifida, and congenital glaucoma, cataract, or sensorineural hearing loss; and immune disorders such as Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, 15 amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, lupus erythematosus, multiple 20 sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, ulcerative colitis, Werner syndrome, and complications of cancer, hemodialysis, and extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and helminthic 25 infections; and trauma. The polynucleotide sequences encoding UBCLE may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and ELISA assays; and in microarrays utilizing fluids or tissues from patient biopsies to detect altered UBCLE expression. Such qualitative or quantitative methods are well known in the art.

30 In a particular aspect, the nucleotide sequences encoding UBCLE may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding UBCLE may be labeled by standard methods and

added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the patient sample is significantly altered from that of a comparable control sample, the 5 nucleotide sequences have hybridized with nucleotide sequences in the sample, and the presence of altered levels of nucleotide sequences encoding UBCLE in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an individual patient.

10        In order to provide a basis for the diagnosis of a disorder associated with expression of UBCLE, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding UBCLE, under conditions suitable for hybridization or amplification. Standard 15 hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

20        Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

25        With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby 30 preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding UBCLE may involve the use of PCR. These oligomers may be chemically

synthesized, generated enzymatically, or produced *in vitro*. Oligomers will preferably contain a fragment of a polynucleotide encoding UBCL, or a fragment of a polynucleotide complementary to the polynucleotide encoding UBCL, and will be employed under optimized conditions for identification of a specific gene or condition.

5 Oligomers may also be employed under less stringent conditions for detection or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of UBCL include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (Melby, P.C. et al. (1993) *J. Immunol.*

10 Methods 159:235-244, and Duplaa, C. et al. (1993) *Anal. Biochem.* 229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of  
15 the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously (to produce a transcript image) and to identify genetic variants, mutations, and polymorphisms. This information may be used in determining gene function, in understanding the genetic basis of a disorder, in diagnosing a disorder, and in developing  
20 and monitoring the activities of therapeutic agents.

In one embodiment, the microarray is prepared and used according to methods known in the art, such as those described in published PCT application WO95/11995 (Chee et al.), Lockhart, D. J. et al. (1996; *Nat. Biotech.* 14:1675-1680), and Schena, M. et al. (1996; *Proc. Natl. Acad. Sci.* 93:10614-10619).

25 The microarray is preferably composed of a large number of unique single-stranded nucleic acid sequences, usually either synthetic antisense oligonucleotides or fragments of cDNAs, fixed to a solid support. The oligonucleotides are preferably about 6 to 60 nucleotides in length, more preferably about 15 to 30 nucleotides in length, and most preferably about 20 to 25 nucleotides in length. For a certain type of microarray, it may be  
30 preferable to use oligonucleotides which are about 7 to 10 nucleotides in length. The microarray may contain oligonucleotides which cover the known 5' or 3' sequence, or may contain sequential oligonucleotides which cover the full length sequence or unique

oligonucleotides selected from particular areas along the length of the sequence.

Polynucleotides used in the microarray may be oligonucleotides specific to a gene or genes of interest in which at least a fragment of the sequence is known or oligonucleotides specific to one or more unidentified cDNAs common to a particular cell or tissue type or 5 to a normal, developmental, or disease state. In certain situations, it may be appropriate to use pairs of oligonucleotides on a microarray. The pairs will be identical, except for one nucleotide preferably located in the center of the sequence. The second oligonucleotide in the pair (mismatched by one) serves as a control. The number of oligonucleotide pairs may range from about 2 to 1,000,000.

10 In order to produce oligonucleotides to a known sequence for a microarray, the gene of interest is examined using a computer algorithm which starts at the 5' end, or, more preferably, at the 3' end of the nucleotide sequence. The algorithm identifies oligomers of defined length that are unique to the gene, have a GC content within a range suitable for hybridization, and lack predicted secondary structure that may interfere with 15 hybridization. In one aspect, the oligomers are synthesized at designated areas on a substrate using a light-directed chemical process. The substrate may be paper, nylon, any other type of membrane, filter, chip, glass slide, or any other suitable solid support.

In one aspect, the oligonucleotides may be synthesized on the surface of the substrate by using a chemical coupling procedure and an ink jet application apparatus, 20 such as that described in published PCT application WO95/251116 (Baldeschweiler et al.). In another aspect, a grid array analogous to a dot or slot blot (HYBRIDOT® apparatus, GIBCO/BRL) may be used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system or thermal, UV, mechanical or chemical bonding procedures. In yet another aspect, an array may be produced by hand or by using 25 available devices, materials, and machines (including Brinkmann® multichannel pipettors or robotic instruments), and may contain 8, 24, 96, 384, 1536, or 6144 oligonucleotides, or any other multiple from 2 to 1,000,000 which lends itself to the efficient use of commercially available instrumentation.

In order to conduct sample analysis using the microarrays, polynucleotides are 30 extracted from a biological sample. The biological samples may be obtained from any bodily fluid (blood, urine, saliva, phlegm, gastric juices, etc.), cultured cells, biopsies, or other tissue preparations. To produce probes, the polynucleotides extracted from the

sample are used to produce nucleic acid sequences which are complementary to the nucleic acids on the microarray. If the microarray consists of cDNAs, antisense RNAs (aRNA) are appropriate probes. Therefore, in one aspect, mRNA is used to produce cDNA which, in turn and in the presence of fluorescent nucleotides, is used to produce 5 fragment or oligonucleotide aRNA probes. These fluorescently labeled probes are incubated with the microarray so that the probe sequences hybridize to the cDNA oligonucleotides of the microarray. In another aspect, nucleic acid sequences used as probes can include polynucleotides, fragments, and complementary or antisense sequences produced using restriction enzymes, PCR technologies, and Oligolabeling or TransProbe 10 kits (Pharmacia & Upjohn) well known in the area of hybridization technology.

Incubation conditions are adjusted so that hybridization occurs with precise complementary matches or with various degrees of less complementarity. After removal of nonhybridized probes, a scanner is used to determine the levels and patterns of fluorescence. The scanned images are examined to determine the degree of 15 complementarity and the relative abundance of each oligonucleotide sequence on the microarray. A detection system may be used to measure the absence, presence, and amount of hybridization for all of the distinct sequences simultaneously. This data may be used for large scale correlation studies or for functional analysis of the sequences, mutations, variants, or polymorphisms among samples. (Heller, R.A. et al. (1997) Proc. 20 Natl. Acad. Sci. 94:2150-2155.)

In another embodiment of the invention, nucleic acid sequences encoding UBCLE may be used to generate hybridization probes useful for mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, such as 25 human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries, such as those reviewed in Price, C.M. (1993; Blood Rev. 7:127-134) and Trask, B.J. (1991; Trends Genet. 7:149-154).

Fluorescent in situ hybridization (FISH, as described in Verma et al. (1988) Human 30 Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, NY) may be correlated with other physical chromosome mapping techniques and genetic map data. Examples of genetic map data can be found in various scientific journals or at the Online

Mendelian Inheritance in Man (OMIM) site. Correlation between the location of the gene encoding UBCLE on a physical chromosomal map and a specific disorder, or predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the subject invention may be used to detect 5 differences in gene sequences between normal, carrier, and affected individuals.

*In situ* hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or 10 arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti, R.A. 15 et al. (1988) *Nature* 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, UBCLE, its catalytic or immunogenic 20 fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between UBCLE and the agent being tested may be measured.

25 Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in published PCT application WO84/03564. In this method, large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with UBCLE, or 30 fragments thereof, and washed. Bound UBCLE is then detected by methods well known in the art. Purified UBCLE can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can

be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding UBCLE specifically compete with a test compound for binding UBCLE. In this manner, antibodies can be used to detect the 5 presence of any peptide which shares one or more antigenic determinants with UBCLE.

In additional embodiments, the nucleotide sequences which encode UBCLE may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base 10 pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

## EXAMPLES

### 15 I. ADRETUT05 cDNA Library Construction

The ADRETUT05 cDNA library was constructed from tumor tissue obtained from the right adrenal gland of a 52 year-old female during a unilateral adrenalectomy. Pathology indicated a pheochromocytoma. Patient history included benign hypertension, depressive disorder, chronic sinusitis, idiopathic proctocolitis, urinary tract infection and 20 irritable colon. Family history included benign hypertension in a sibling, cerebrovascular disease, secondary Parkinsonism and irritable colon in the mother; atherosclerotic coronary artery disease, hyperlipidemia and malignant brain neoplasm in siblings and secondary Parkinsonism in the father.

The frozen tissues were homogenized and lysed in Trizol reagent (1 gm tissue/10 25 ml Trizol; Cat. #10296-028; Gibco/BRL), a monoplastic solution of phenol and guanidine isothiocyanate, using a Brinkmann Homogenizer Polytron PT-3000 (Brinkmann Instruments, Westbury, NY). After a brief incubation on ice, chloroform was added (1:5 v/v), and the lysate was centrifuged. The upper chloroform layer was removed to a fresh tube and the RNA extracted with isopropanol, resuspended in DEPC-treated water, and 30 DNase treated for 25 min at 37°C. The RNA was re-extracted once with acid phenol-chloroform pH 4.7 and precipitated using 0.3M sodium acetate and 2.5 volumes ethanol. The mRNA was then isolated with the Qiagen Oligotex kit (QIAGEN, Inc.,

Chatsworth, CA) and used to construct the cDNA library.

The mRNA was handled according to the recommended protocols in the SuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning (Cat. #18248-013, Gibco/BRL). The cDNAs were fractionated on a Sepharose CL4B column (Cat. #275105-01; Pharmacia), and those cDNAs exceeding 400 bp were ligated into pINCY 1. The plasmid pINCY 1 was subsequently transformed into DH5a™ competent cells (Cat. #18258-012; Gibco/BRL).

## II Isolation and Sequencing of cDNA Clones

Plasmid DNA was released from the cells and purified using the REAL Prep 96 Plasmid Kit (Catalog #26173, QIAGEN). The recommended protocol was employed except for the following changes: 1) the bacteria were cultured in 1 ml of sterile Terrific Broth (Catalog #22711, Gibco/BRL) with carbenicillin at 25 mg/L and glycerol at 0.4%; 2) the cultures were incubated for 19 hours after the wells were inoculation and then lysed with 0.3 ml of lysis buffer; 3) following isopropanol precipitation, the DNA pellet was resuspended in 0.1 ml of distilled water. After the last step in the protocol, samples were transferred to a 96-well block for storage at 4° C.

The cDNAs were sequenced by the method of Sanger et al. (1975, J. Mol. Biol. 94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno, NV) in combination with Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown, MA) and Applied Biosystems 377 DNA Sequencing Systems.

## III. Homology Searching of cDNA Clones and Their Deduced Proteins

The nucleotide sequences and/or amino acid sequences of the Sequence Listing were used to query sequences in the GenBank, SwissProt, BLOCKS, and Pima II databases. These databases, which contain previously identified and annotated sequences, were searched for regions of homology using BLAST (Basic Local Alignment Search Tool). (Altschul, S.F. (1993) J. Mol. Evol 36:290-300; and Altschul et al. (1990) J. Mol. Biol. 215:403-410.)

BLAST produced alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST was

especially useful in determining exact matches or in identifying homologs which may be of prokaryotic (bacterial) or eukaryotic (animal, fungal, or plant) origin. Other algorithms such as the one described in Smith, T. et al. (1992; Protein Engineering 5:35-51), could have been used when dealing with primary sequence patterns and secondary structure gap 5 penalties. The sequences disclosed in this application have lengths of at least 49 nucleotides and have no more than 12% uncalled bases (where N is recorded rather than A, C, G, or T).

The BLAST approach searched for matches between a query sequence and a database sequence. BLAST evaluated the statistical significance of any matches found, 10 and reported only those matches that satisfy the user-selected threshold of significance. In this application, threshold was set at  $10^{-25}$  for nucleotides and  $10^{-10}$  for peptides.

Incyte nucleotide sequences were searched against the GenBank databases for primate (pri), rodent (rod), and other mammalian sequences (mam), and deduced amino acid sequences from the same clones were then searched against GenBank functional 15 protein databases, mammalian (mamp), vertebrate (vrtp), and eukaryote (eukp), for homology.

#### IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a 20 transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (Sambrook, *supra*).

Analogous computer techniques applying BLAST are used to search for identical or related molecules in nucleotide databases such as GenBank or the LIFESEQ™ database 25 (Incyte Pharmaceuticals). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or homologous.

The basis of the search is the product score, which is defined as:

% sequence identity x % maximum BLAST score

30

100

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match

will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Homologous molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analysis are reported as a list of libraries in which the transcript encoding UBCLE occurs. Abundance and percent abundance are also reported. 5 Abundance directly reflects the number of times a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences examined in the cDNA library.

#### 10 V. Extension of UBCLE Encoding Polynucleotides

The nucleic acid sequence of Incyte Clone 2501808 was used to design oligonucleotide primers for extending a partial nucleotide sequence to full length. One primer was synthesized to initiate extension in the antisense direction, and the other was synthesized to extend sequence in the sense direction. Primers were used to facilitate the 15 extension of the known sequence "outward" generating amplicons containing new unknown nucleotide sequence for the region of interest. The initial primers were designed from the cDNA using OLIGO 4.06 (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. 20 Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries (GIBCO/BRL) were used to extend the sequence. If more than one extension is necessary or desired, additional sets of primers are designed to further extend the known region.

25 High fidelity amplification was obtained by following the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix. PCR was performed using the Peltier Thermal Cycler (PTC200; M.J. Research, Watertown, MA), beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, with the following parameters:

30	Step 1	94° C for 1 min (initial denaturation)
	Step 2	65° C for 1 min
	Step 3	68° C for 6 min
	Step 4	94° C for 15 sec

Step 5	65° C for 1 min
Step 6	68° C for 7 min
Step 7	Repeat steps 4 through 6 for an additional 15 cycles
Step 8	94° C for 15 sec
5 Step 9	65° C for 1 min
Step 10	68° C for 7:15 min
Step 11	Repeat steps 8 through 10 for an additional 12 cycles
Step 12	72° C for 8 min
Step 13	4° C (and holding)

10 A 5  $\mu$ l to 10  $\mu$ l aliquot of the reaction mixture was analyzed by electrophoresis on a low concentration (about 0.6% to 0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products were excised from the gel, purified using QIAQuick™ (QIAGEN), and trimmed of 15 overhangs using Klenow enzyme to facilitate religation and cloning.

After ethanol precipitation, the products were redissolved in 13  $\mu$ l of ligation buffer, 1  $\mu$ l T4-DNA ligase (15 units) and 1  $\mu$ l T4 polynucleotide kinase were added, and the mixture was incubated at room temperature for 2 to 3 hours, or overnight at 16° C. Competent E. coli cells (in 40  $\mu$ l of appropriate media) were transformed with 3  $\mu$ l of 20 ligation mixture and cultured in 80  $\mu$ l of SOC medium. (Sambrook, *supra*). After incubation for one hour at 37° C, the E. coli mixture was plated on Luria Bertani (LB) agar (Sambrook, *supra*) containing 2x Carb. The following day, several colonies were randomly picked from each plate and cultured in 150  $\mu$ l of liquid LB/2x Carb medium placed in an individual well of an appropriate commercially-available sterile 96-well 25 microtiter plate. The following day, 5  $\mu$ l of each overnight culture was transferred into a non-sterile 96-well plate and, after dilution 1:10 with water, 5  $\mu$ l from each sample was transferred into a PCR array.

For PCR amplification, 18  $\mu$ l of concentrated PCR reaction mix (3.3x) containing 4 units of rTh DNA polymerase, a vector primer, and one or both of the gene specific 30 primers used for the extension reaction were added to each well. Amplification was performed using the following conditions:

Step 1	94° C for 60 sec
Step 2	94° C for 20 sec
Step 3	55° C for 30 sec
35 Step 4	72° C for 90 sec
Step 5	Repeat steps 2 through 4 for an additional 29 cycles
Step 6	72° C for 180 sec

Step 7                    4° C (and holding)

Aliquots of the PCR reactions were run on agarose gels together with molecular weight markers. The sizes of the PCR products were compared to the original partial 5 cDNAs, and appropriate clones were selected, ligated into plasmid, and sequenced.

In like manner, the nucleotide sequence of SEQ ID NO:2 is used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for 5' extension, and an appropriate genomic library.

#### 10 VI. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:2 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with 15 larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 (National Biosciences) and labeled by combining 50 pmol of each oligomer and 250  $\mu$ Ci of [ $\gamma$ -<sup>32</sup>P] adenosine triphosphate (Amersham) and T4 polynucleotide kinase (DuPont NEN®, Boston, MA). The labeled oligonucleotides are substantially purified using a Sephadex G-25 superfine resin column (Pharmacia & 20 Upjohn). An aliquot containing 10<sup>7</sup> counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN®).

The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham, NH).

25 Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR™ film (Kodak, Rochester, NY) is exposed to the blots in a Phosphoimager cassette (Molecular Dynamics, Sunnyvale, CA) for several hours, hybridization patterns are compared 30 visually.

#### VII. Micr arrays

To produce oligonucleotides for a microarray, one of the nucleotide sequences of the present invention is examined using a computer algorithm which starts at the 3' end of the nucleotide sequence. The algorithm identifies oligomers of defined length that are unique to the gene, have a GC content within a range suitable for hybridization, and lack 5 predicted secondary structure that would interfere with hybridization. The algorithm identifies approximately 20 sequence-specific oligonucleotides of 20 nucleotides in length (20-mers). A matched set of oligonucleotides are created in which one nucleotide in the center of each sequence is altered. This process is repeated for each gene in the microarray, and double sets of twenty 20-mers are synthesized and arranged on the surface 10 of the silicon chip using a light-directed chemical process, such as that described in Chee 15 (supra).

In the alternative, a chemical coupling procedure and an ink jet device are used to synthesize oligomers on the surface of a substrate. (See Baldeschweiler, supra.) In another alternative, a grid array analogous to a dot or slot blot is used to arrange and link 15 cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system or thermal, UV, mechanical, or chemical bonding procedures. A typical array may be produced by hand or using available materials and machines and contain grids of 8 dots, 24 dots, 96 dots, 384 dots, 1536 dots, or 6144 dots. After hybridization, the microarray is washed to remove nonhybridized probes, and a scanner is used to determine the levels and 20 patterns of fluorescence. The scanned image is examined to determine the degree of complementarity and the relative abundance/expression level of each oligonucleotide sequence in the microarray.

### VIII. Complementary Polynucleotides

25 Sequences complementary to the UBCLE-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring UBCLE. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using Oligo 4.06 software and the coding 30 sequence of UBCLE. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to

prevent ribosomal binding to the UBCLE-encoding transcript.

#### **IX. Expression of UBCLE**

Expression of UBCLE is accomplished by subcloning the cDNAs into appropriate vectors and transforming the vectors into host cells. In this case, the cloning vector is also used to express UBCLE in *E. coli*. This vector contains a promoter for  $\beta$ -galactosidase upstream of the cloning site, followed by sequence containing the amino-terminal Met and the subsequent seven residues of  $\beta$ -galactosidase. Immediately following these eight residues is a bacteriophage promoter useful for transcription and a linker containing a number of unique restriction sites.

Induction of an isolated, transformed bacterial strain with isopropyl beta-D-thiogalactopyranoside (IPTG) using standard methods produces a fusion protein which consists of the first 8 residues of  $\beta$ -galactosidase, about 5 to 15 residues of linker, and the full length protein. The signal residues direct the secretion of UBCLE into bacterial growth media which can be used directly in the following assay for activity.

#### **X. Demonstration of UBCLE Activity**

UBCLE activity is demonstrated by the formation of di-ubiquitin conjugates from free ubiquitin (van Nocker et al. *supra*). UBCLE is incubated together with 75 pmol  $^{125}$ I-labeled ubiquitin, 20 nM wheat E1, 2 mM Mg ATP, 0.1 mM dithiothreitol, and 50 mM Tris-HCl, pH 8.0. The reaction is incubated for 2 minutes at 4°C and the di-ubiquitin product separated from free ubiquitin by polyacrylamide gel electrophoresis. Di-ubiquitin is visualized by autoradiography, removed from the gel, and counted in a gamma radioisotope counter. The amount of di-ubiquitin formed in the reaction is proportional to the activity of UBCLE in the assay.

#### **XI. Production of UBCLE Specific Antibodies**

UBCLE substantially purified using PAGE electrophoresis (Sambrook, *supra*), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols. The UBCLE amino acid sequence is analyzed using DNASTAR software (DNASTAR Inc) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to

those of skill in the art. Selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions, is described by Ausubel (*supra*) and by others.

Typically, the oligopeptides are 15 residues in length, and are synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and 5 coupled to KLH (Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), following the procedure described in Ausubel et al., *supra*. Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and 10 reacting with radio-iodinated goat anti-rabbit IgG.

## **XII. Purification of Naturally Occurring UBCLE Using Specific Antibodies**

Naturally occurring or recombinant UBCLE is substantially purified by immunoaffinity chromatography using antibodies specific for UBCLE. An 15 immunoaffinity column is constructed by covalently coupling UBCLE antibody to an activated chromatographic resin, such as CNBr-activated Sepharose (Pharmacia & Upjohn). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing UBCLE are passed over the immunoaffinity column, and the 20 column is washed under conditions that allow the preferential absorbance of UBCLE (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/UBCLE binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and UBCLE is collected.

## **25 XIII. Identification of Molecules Which Interact with UBCLE**

UBCLE or biologically active fragments thereof are labeled with  $^{125}\text{I}$  30 Bolton-Hunter reagent. (Bolton et al. (1973) Biochem. J. 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled UBCLE, washed, and any wells with labeled UBCLE complex are assayed. Data obtained using different concentrations of UBCLE are used to calculate values for the number, affinity, and association of UBCLE with the candidate molecules.

Various modifications and variations of the described methods and systems of the

invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications 5 of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

What is claimed is:

1. A substantially purified ubiquitin-like conjugating protein (UBCLE) comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.  
5
2. A substantially purified variant of UBCLE having at least 90% amino acid identity to the amino acid sequence of claim 1.
3. An isolated and purified polynucleotide sequence encoding the UBCLE of  
10 claim 1.
4. An isolated and purified polynucleotide variant having at least 90% polynucleotide identity to the polynucleotide sequence of claim 3.
- 15 5. A composition comprising the polynucleotide sequence of claim 3.
6. An isolated and purified polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence of claim 3.
- 20 7. An isolated and purified polynucleotide sequence which is complementary to the polynucleotide sequence of claim 3.
8. An isolated and purified polynucleotide sequence comprising SEQ ID NO:2 or a fragment of SEQ ID NO:2.  
25
9. An isolated and purified polynucleotide variant having at least 90% polynucleotide identity to the polynucleotide sequence of claim 8.
10. An isolated and purified polynucleotide sequence which is complementary  
30 to the polynucleotide sequence of claim 8.
11. An expression vector containing at least a fragment of the polynucleotide

sequence of claim 3.

12. A host cell containing the expression vector of claim 11.

5 13. A method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, the method comprising the steps of:

- (a) culturing the host cell of claim 12 under conditions suitable for the expression of the polypeptide; and
- (b) recovering the polypeptide from the host cell culture.

10

14. A pharmaceutical composition comprising the UBCLE of claim 1 in conjunction with a suitable pharmaceutical carrier.

15. A purified antibody which specifically binds to the UBCLE of claim 1.

15

16. A purified agonist of the UBCLE of claim 1.

17. A purified antagonist of the UBCLE of claim 1.

20 18. A method for treating or preventing cancer, the method comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 17.

25 19. A method for treating or preventing a developmental disorder, the method comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 17.

30 20. A method for treating or preventing an immune disorder the method comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 17.

21. A method for treating or preventing a neuronal disorder the method

comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 17.

22. A method for detecting a polynucleotide encoding UBCLE in a biological  
5 sample containing nucleic acids, the method comprising the steps of:

- (a) hybridizing the polynucleotide of claim 7 to at least one of the nucleic acids of the biological sample, thereby forming a hybridization complex; and
- (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide encoding UBCLE in the biological sample.

10 23. The method of claim 22 wherein the nucleic acids of the biological sample are amplified by the polymerase chain reaction prior to the hybridizing step.

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CG	TTC	CTG	CGT	TGT	GAG	GGG	TGT	CAG	CTC	AGT	GCA	TCC	CAG	GCA	GCT	CTT	AGT
9	18	27	36	45	54												
CGT	GAG	CAG	TGA	ACT	GTG	TGT	GGT	TCC	TTC	TAC	TGT	GGG	ATC	ATG	CAG	AGA	GCT
63	72	81	90	99	108												
S	R	L	K	R	E	L	H	M	L	A	T	E	P	P	G	I	
117	126	135	144	153	162												
TCA	TGT	CTG	AAG	AGA	GAG	CTG	CAC	ATG	TTA	GCC	ACA	GAG	CCA	CCC	CCA	GGC	ATC
T	C	W	Q	D	K	D	Q	M	D	D	D	R	A	Q	I	L	G
171	180	189	198	207	216												
ACA	TGT	TGG	CAA	GAT	AAA	GAC	CAA	ATG	GAT	GAC	CTG	CGA	GCT	CAA	ATA	TAA	GGT
G	A	A	N	T	P	Y	E	K	G	V	F	K	L	E	V	I	P
225	234	243	252	261	270												
GGA	GCC	AAC	ACA	CCT	TAT	GAG	AAA	GGT	GTT	TTT	AAG	CTA	GAA	GTT	ATC	ATT	CCT
G	A	A	N	T	P	Y	E	K	G	V	F	K	L	E	V	I	P
279	288	297	306	315	324												
GAG	AGG	TAC	CCA	TTT	GAA	CCT	CCT	CAG	ATC	CGA	TTT	CTC	ACT	CCA	ATT	TAT	CAT
E	R	Y	P	F	E	P	P	Q	I	R	F	L	T	P	I	Y	H
333	342	351	360	369	378												
CCA	AAC	ATT	GAT	TCT	GCT	GGA	AGG	ATT	TGT	CTG	GAT	GTT	CTC	AAA	TTG	CCA	CCA
P	N	I	D	S	A	G	R	I	C	L	D	V	L	K	L	P	P

FIGURE 1A

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A	AA	GGT	GCT	TGG	AGA	CCA	TCC	CTC	AAC	ATC	GCA	ACT	GTG	T <sup>r</sup> TG	ACC	TCT	ATT	CAG
K	G	A	W	R	P	S	L	N	I	A	T	V	L	T	S	I	Q	
441	450				459				468				477				486	
CTG	CTC	ATG	TCA	GAA	CCC	AAC	CCT	GAT	GAC	CCG	CTC	ATG	GCT	GAC	ATA	TCC	TCA	
L	L	M	S	E	P	N	P	D	D	P	L	M	A	D	I	S	S	
495	504				513				522				531				540	
GAA	TTT	AAA	TAT	AAT	AAG	CCA	GCC	TTC	CTC	AAG	AAT	GCC	AGA	CAG	TGG	ACA	GAG	
E	F	K	Y	N	K	P	A	F	L	K	N	A	R	Q	W	T	E	
549	558				567				576				585				594	
AAG	CAT	GCA	AGA	CAG	AAA	CAA	AAG	GCT	GAT	GAG	GAA	GAG	ATG	C <sup>r</sup> T	GAT	AAT	CTA	
K	H	A	R	Q	K	Q	K	A	D	E	E	E	M	L	D	N	L	
603	612				621				630				639				648	
CCA	GAG	GCT	GGT	GAC	TCC	AGA	GTA	CAC	AAC	TCA	ACA	CAG	AAA	AGG	AAG	GCC	AGT	
P	E	A	G	D	S	R	V	H	N	S	T	Q	K	R	K	A	S	
657	666				675				684				693				702	
CAG	CTA	GTA	GGC	ATA	GAA	AAG	AAA	TTT	CAT	CCT	GAT	GTT	TAG	GGG	ACT	TGT	CCT	
Q	L	V	G	I	E	K	K	F	H	P	D	V						
711	720				729				738				747				756	
GGT	TCA	TCT	TAG	TTA	ATG	TGT	TCT	TTG	CCA	AGG	TGA	TCT	AAG	TTG	CCT	ACC	TTG	

FIGURE 1B

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765      774      783      792      801      810  
AAT TTT TTA AAT ATA TTT GAT GAC ATA ATT TTT GTG TAG TTT ATT TAT CTT

819      828      837      846      855      864  
GTA CAT ATG TAT TTT GAA ATC TTT TAA ACC TGA AAA ATA AAT AGT CAT TTA ATG

873  
TTG AAA AAA AAA AA

FIGURE 1C

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1 [M] Q R A S R L K R E L H M L A T E P P G I T C W Q D K D Q 2501808  
 1 [M] A - L K R I N K E L L S D L A R D P P A Q C S A G P V G D D GI 1145691  
  
 31 [M] D D L R A O I L G G A N T P Y E K G V F K L E V I I P E R 2501808  
 30 [M] F H W Q [A] T I M G P N D S P Y Q G G V F F L T I H F P T D GI 1145691  
  
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 60 [Y] P F E P K P V A F T T R I Y H P N I N S N G S I C L D T I L GI 1145691  
  
 91 [K] L P P K G A W R P S L N I A T V L T S I Q L L M S E P N P 2501808  
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 121 [D] D P L M A D I S S E F K Y N K P A F L K N A R Q W T E K H 2501808  
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 181 [R] K A S Q L V G I E K F H P D V 2501808  
 147 - M GI 1145691

FIGURE 2

## SEQUENCE LISTING

<110> INCYTE PHARMACEUTICALS, INC.  
HILLMAN, Jennifer L.  
SHAH, Purvi  
CORLEY, Neil C.

<120> UBIQUITIN-LIKE CONJUGATING PROTEIN

<130> PF-0439 PCT

<140> To Be Assigned  
<141> Herewith

<150> 08/989,289

<151> 1997-12-12

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35 40 45  
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50 55 60  
Tyr Pro Phe Glu Pro Pro Gln Ile Arg Phe Leu Thr Pro Ile Tyr  
65 70 75  
His Pro Asn Ile Asp Ser Ala Gly Arg Ile Cys Leu Asp Val Leu  
80 85 90  
Lys Leu Pro Pro Lys Gly Ala Trp Arg Pro Ser Leu Asn Ile Ala  
95 100 105  
Thr Val Leu Thr Ser Ile Gln Leu Leu Met Ser Glu Pro Asn Pro  
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Asp Asp Pro Leu Met Ala Asp Ile Ser Ser Glu Phe Lys Tyr Asn  
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140 145 150  
Ala Arg Gln Lys Gln Lys Ala Asp Glu Glu Glu Met Leu Asp Asn  
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acccaaatgga	tgacacctgca	gctcaaataat	taggtggagc	caacacacccat	tatgagaaaag	240	
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Phe His Trp Gln Ala Thr Ile Met Gly Pro Asn Asp Ser Pro Tyr
35 40 45
Gln Gly Gly Val Phe Phe Leu Thr Ile His Phe Pro Thr Asp Tyr
50 55 60
Pro Phe Lys Pro Pro Lys Val Ala Phe Thr Thr Arg Ile Tyr His
65 70 75
Pro Asn Ile Asn Ser Asn Gly Ser Ile Cys Leu Asp Ile Leu Arg
80 85 90
Ser Gln Trp Ser Pro Ala Leu Thr Ile Ser Lys Val Leu Leu Ser
95 100 105
Ile Cys Ser Leu Leu Cys Asp Pro Asn Pro Asp Asp Pro Leu Val
110 115 120
Pro Glu Ile Ala Arg Ile Tyr Lys Thr Asp Arg Asp Lys Tyr Asn
125 130 135
Arg Ile Ser Arg Glu Trp Thr Gln Lys Tyr Ala Met
140 145

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/25564

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 6 C12N15/52 C12N5/10 C12N1/21 C12N9/00 C12Q1/68  
C07K16/40 A61K38/53

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12Q C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMEST15 E.M.B.L. Databases Accession Number: AA046871, 7 September 1996</p> <p>HILLIER L ET AL: "Homo sapiens cDNA clone 376769 5' similar to SW:UBC5_DROME P35128 UBIQUITIN-CONJUGATING ENZYME E2-17KD" XP002101053</p> <p>98.7% identity in 521 bp overlap see abstract</p> <p>---</p>	1-13
Y	<p>---</p> <p>-/-</p>	14-23

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

23 April 1999

Date of mailing of the international search report

07/05/1999

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Lonnoy, O

## INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/25564

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMEST15 E.M.B.L. Databases Accession Number: AA046828, 7 September 1996 HILLIER L ET AL: "Homo sapiens cDNA clone 376769 3' similar to SW:UBC5_DROME P35128 UBIQUITIN-CONJUGATING ENZYME E2-17KD" XP002101054 99.4% identity in 522bp overlap see abstract ----	1-13
Y	99.4% identity in 522bp overlap see abstract	14-23
X	DATABASE EMEST2 E.M.B.L. Databases Accession Number: AA643701, 1 November 1997 NATIONAL CANCER INSTITUTE: "Homo sapiens cDNA clone IMAGE:1058538 similar to SW:UBC5_DROME P35128 UBIQUITIN-CONJUGATING ENZYME E2-17KD" XP002101055 98.5% identity in 470bp overlap see abstract ----	1-13
Y	98.5% identity in 470bp overlap see abstract	14-23
X	DATABASE EMEST12 E.M.B.L. Databases Accession Number: AA505790, 4 July 1997 NATIONAL CANCER INSTITUTE: "Homo sapiens cDNA clone IMAGE:966527 similar to SW:UBC5_DROME P35128 UBIQUITIN-CONJUGATING ENZYME E2-17KD" XP002101056 99.5% identity in 437bp overlap see abstract ----	1-13
Y	99.5% identity in 437bp overlap see abstract	14-23
Y	WO 96 23410 A (HUMAN GENOME SCIENCES INC ;NI JIAN (US); GENTZ REINER (US); ADAMS) 8 August 1996 see abstract ----	14-23
Y	WO 95 18974 A (MITOTIX INC) 13 July 1995 see abstract ----	14-23
Y	WO 94 17816 A (HARVARD COLLEGE ;DANA FARBER CANCER INST INC (US)) 18 August 1994 see abstract ----	14-23
		-/-

## INTERNATIONAL SEARCH REPORT

Interr	nal Application No
PCT/US 98/25564	

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE GENESEQ            Accession Number: T19730, 5 July 1996            MATSUBARA K AND OKUBO K: "Human Gene            Signature HUMGS00804"            XP002101057            99.3% identity in 134bp overlap            see abstract            &amp; WO 95 14772 A (MATSUBARA K AND OKUBO K)            1 June 1995            see abstract</p> <p>---</p>	4-10
A	<p>JENSEN J ET AL: "Identification of a            family of closely related human ubiquitin            conjugating enzymes"            JOURNAL OF BIOLOGICAL CHEMISTRY.,            vol. 270, 1995, pages 30408-30414,            XP002101052            cited in the application            see abstract</p> <p>---</p>	
A	<p>EP 0 626 450 A (WISCONSIN ALUMNI RES            FOUND) 30 November 1994            see abstract</p> <p>-----</p>	

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Although claims 18-21 are directed to methods of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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Claims 16 and 17, respectively relating to an agonist and an antagonist of the UBCLE of claim 1, could not be searched completely as their subject-matters were not sufficiently disclosed.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/US 98/25564

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
WO 9623410	A 08-08-1996	AU 1869095	A	21-08-1996	
		EP 0814661	A	07-01-1998	
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